Unlocking a cure from within for X-linked diseases

The mammalian X chromosome is home to many genes that are important for brain development and function, as well as genes associated with reproduction. More than 200 disease-causing mutations have been identified on the X-chromosome. Diseases caused by a mutation on the X chromosome typically manifest in males, who generally carry only one X chromosome. Females have 2 X chromosomes, but one X chromosome is always silenced by a process called X chromosome inactivation (XCI) in order to balance gene dosage with males. Thus, females are mosaics. Because the choice of which X chromosome occurs randomly, each X chromosome has a ~50% chance of becoming the inactive X (Xi) or the active X (Xa). When a female harbors a mutation on one X chromosome, approximately half of all cells in her body would express the mutation on the Xa. But in those same cells, a normal copy of the gene occurs on the Xi. Therefore, in X-linked diseases such as Rett Syndrome, only half of a female's cells are actually expressing the mutant product. Yet, this situation causes a severe neurodevelopmental disorder that is manifested by seizures and autism. Mutations in the Methyl-CpG binding protein 2 (MECP2), a chromatin-associated gene product, underlie Rett Syndrome. Because disease cells carry a normal copy of the gene on the Xi, every female patient harbors a potential cure within their own cells (Fig. 1). Our major goal has been to reactivate the normal copy on the Xi to stop progression of the disease, or even to reverse it.

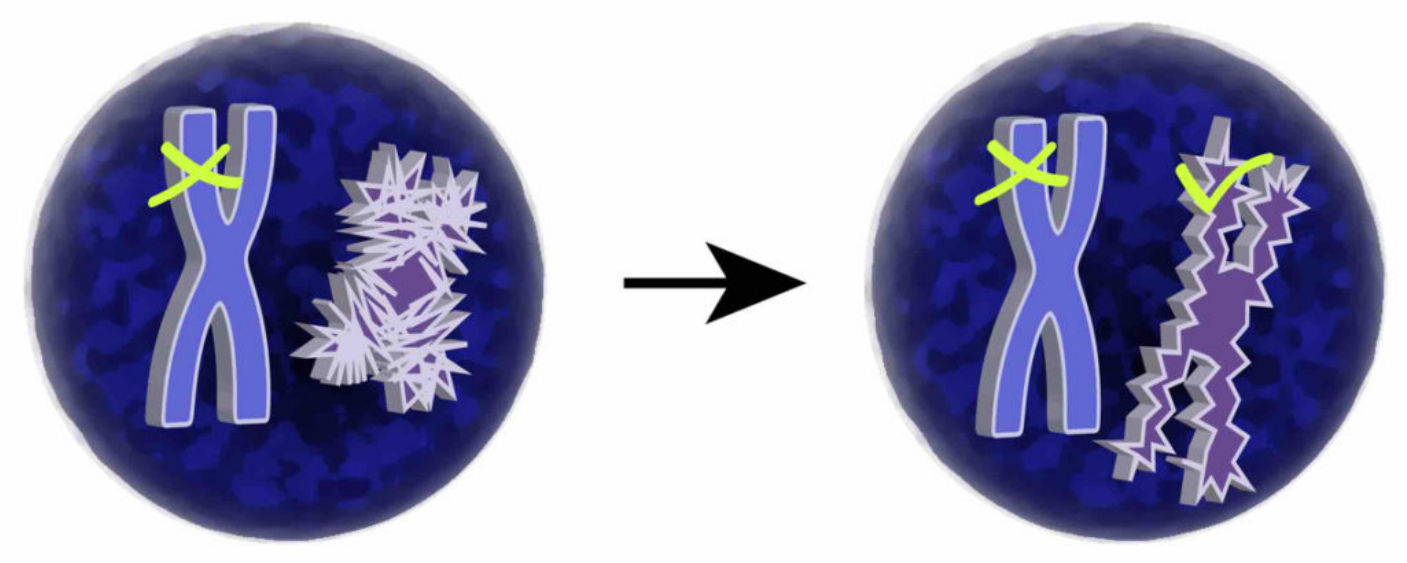

Fig. 1. The principle of Xi-reactivation as a treatment for female X-linked diseases. A female cell with a mutation on the active X chromosome (Xa) contains an inactivated X chromosome (Xi) with a wild type allele. The aim is to leverage the wild type allele by turning it back on to restore expression in patients.

Reactivating the Xi has been difficult to achieve because of many layers of silencing that characterize XCI. In our recent work, however, we were able to identify a cocktail of molecules that succeeded in a partial Xi reactivation. This cocktail is a mixture of a small molecule inhibitor of DNA methylation [5-Aza-2'deoxycytidine (Aza)] and an antisense oligonucleotide (ASO) that degrades an RNA that normally orchestrates XCI. 5-Aza-2'deoxycytidine is an FDA-approved drug, and our goal here is to repurpose it for treatment of X-linked disease. In cells treated for 5 days with our drug cocktail, Mecp2 was reactivated 30,000-fold above the Xi baseline — the equivalent of 3-5% of what is normally expressed on the Xa (Fig. 2). While this degree of reactivation might seem modest, our parallel work in a female Rett Syndrome mouse model suggests that 3-5% expression of MECP2 protein confers a significant degree of phenotypic benefit (https://doi.org/10.1073/pnas.1800931115). Ongoing work is aimed at boosting the degree of reactivation further.

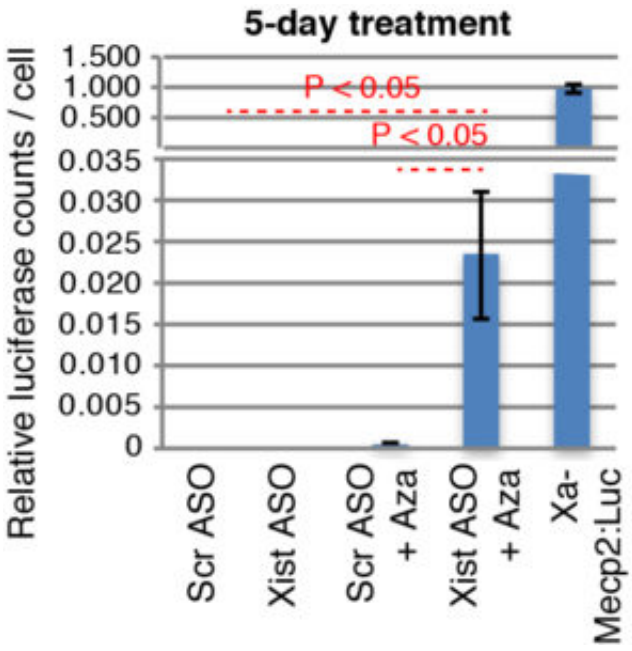

Fig. 2. Luminescence measurements of 5-day treated cells with a luciferase reporter fused to Mecp2 on the Xi (or Xa, control). While Xist ASO and Aza cause hardly any reactivation on their own, together they have a synergistic effect.

Given that sex chromosomal dosage compensation is important during development and Xist expression is maintained throughout female life, one important question is whether loss of Xist expression and the perturbation of XCI would have undesirable side effects. We tested this by looking for consequences of deleting Xist in the brain. We found no significant phenotypic differences between mice with or without Xist in their brains across an entire lifespan. We also mimicked the drug combination treatment in mice by injecting the small molecule Aza into mice lacking Xist in their brains. Analysis of the gene expression in the brain showed the same synergistic effect between loss of Xist and inhibition of DNA methylation. Moreover and importantly, the treatment did not induce observable adverse effects acutely or over the course of a year follow-up. This is good news for the Xi reactivation platform. If successful, the Xi reactivation strategy could be applied to a number of other X-linked neurodevelopmental diseases, including CDKL5 disorder, Fragile X syndrome, and CLCN4 disorder.

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A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders.
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